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# Borax as a Preservative of Dairy Waste for the B.O.D. Test

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In pilot-plant investigations and industrial waste surveys, it is often almost impossible to run B.O.D. tests immediately after sampling. The validity of analyses of samples that must be held for several days or shipped to a laboratory is open to question, especially when a soluble material that is readily attacked by microorganisms makes up a major portion of the oxygen-demanding substances. Previous studies at this laboratory demonstrated that a dairy waste heavily inoculated with aerobic microflora is rapidly oxidized if sufficient oxygen is available. Thus a solution containing 1000 p.p.m. milk solids was about 40 percent oxidized in six hours, with an accompanying loss in B.O.D. of more than 50 percent (2, 3).

Several possible methods of preserving B.O.D. have been studied by other workers. Freezing the sample and holding it in the frozen state is effective but often inconvenient. Use of a completely filled sample container has been investigated by Horton (4), who found that filled bottles of milk waste kept under water showed a negligible loss in B.O.D. over several days. In studies requiring storage of casein solutions at this laboratory, borax had been found effective at a concentration of two percent but completely ineffective at concentrations below one percent. The work reported in this paper, which is of a preliminary nature, was undertaken for the purpose of determining the use of borax as a preservative for dairy wastes.

Borax, sodium tetraborate ($Na_2B_4O_7.10\ H_2O$), consists of colorless, odorless, transparent crystals that have a solubility of one gram in 16 ml. water at room temperature and one gram in 0.6 ml. boiling water. The resulting solution at room temperature is buffered at pH 9.2. Borax is not oxidized under the conditions of the C.O.D. test.

Buchanan and Fulmer (1) in their discussion of effects of chemical environment on the physiology and biochemistry of bacteria report findings of other authors. *Aspergillus* was inhibited by one percent boric

acid, and 0.6 percent inhibited a *Penicillium*. On the other hand, *Eberthella typhosa* required 2.7 percent. A mixture of one part boric acid and three parts borax prevented souring of milk for 24 hours when used in the proportion of 1:2000.

McCulloch (6) states that borax had little germicidal action at normal temperature, but boric acid, a feeble germicide, was somewhat effective as a basteriostat. He refers to other studies wherein one percent boric acid was used to preserve milk samples for shipment to the laboratory for bacteriological examination.

Porter (8) in his discussion on bacteriostasis gives a condensation of the excellent treatise of Marshall and Hrenoff (5), who studied the action of 20 disinfectants on six organisms. There are bands or ranges in which the disinfectants display different overlapping effects. Thus, there is an ineffective band, a stimulative band, an inhibitory band, and a bactericidal band. In various experiments the limits of these bands varied appreciably; consequently, the concentration inhibitory in one experiment might be bactericidal in another. Boric acid exhibited complete inhibition at concentrations of 1-60 to 1-470, but gave a delayed disinfectant action after 24 hours at concentrations of 1-150 to 1-1000; only slight retardation of growth was observed in concentrations of 1-470 to 1-1000. Negative effect on growth of pure cultures was noticed with solutions containing less than 1000 p.p.m., but complete inhibition was assured with 1.67 percent boric acid.

The most recent and thorough review on the antibacterial action of boric acid and boron compounds is that by Novak (7). He evaluated these substances as to their activity on bacteria, fungi and viruses. Among other things, he concluded that the germicidal or killing action does not occur within the time limits designated for the phenol coefficient test. There is a pronounced bacteriostatic action in concentrations of 0.2 to 4.0 percent boric acid, and, if exposure is sufficiently prolonged, these concentrations kill vegetative bacteria.

From the above, it may be inferred that borax is a mild antiseptic agent that has definite preserving ability. Borax is a bacteriostat rather than a bactericide, but upon long exposure may be bactericidal. Hence, the action of borax, as is true of so many other preservatives, is not sharply delineated. Experiments using borax as a preserving agent for a simulated dairy waste consisting of dried skim milk solutions are described.

## *Experimental*

Initial studies were made on odor and turbidity changes in seeded and unseeded solutions of a simulated dairy waste of 0.1 percent dried

skim milk solids protected by borax in concentrations of 0, 0.5, 1.0 and 2.0 percent. After seven days' exposure at room temperature, the 0.5 percent concentration of borax proved completely ineffective; the one percent concentration was not wholly effective; but the two percent borax solution prevented both odor and visible bacterial growth. After ten days' exposure, a chemical oxygen demand determination of this two percent borated milk solution showed only 12 percent reduction as compared with the C.O.D. of the original uninoculated solution. Reductions of 45 to 70 percent occurred in unseeded and seeded samples of one percent borax content, respectively, giving further proof of the ineffectualness of the preserving action at this low concentration.

Detailed studies on changes in oxygen demand, both biological and chemical, in the presence of borax were then made. Five hundred milliliter quantities of 1000 p.p.m. skim milk were distributed into liter flasks. To half the flasks, 10 gram amounts of borax were added. All flasks were inoculated with a mixed culture from an aerator tank and exposed at room temperature. Determinations for C.O.D. and B.O.D. were made as recorded in Table I.

TABLE I

PRESERVATIVE ACTION OF TWO PERCENT BORATED WASTE AS MEASURED BY B.O.D. AND C.O.D.

| Days | B.O.D. p.p.m. | | C.O.D. p.p.m. | |
|---|---|---|---|---|
| | No Borax | 2% Borax | No Borax | 2% Borax |
| 0 | 554 | 540 | 977 | 984 |
| 2 | 432 | 548 | 519 | 942 |
| 7 | 183 | 538 | 260 | 936 |

As expected, samples without borax spoiled. A 70 percent reduction in both C.O.D. and B.O.D. had occurred after 7 days. Even within two days, substantial reductions were shown in the non-borated samples. In the borax-treated wastes, however, activity was practically stopped. The pH of this material was 9.25 at the start and 9.15 at the end, whereas the pH of the untreated waste first dropped to 6.4 and then became alkaline at pH 8.25, indicating an initial acid production from lactose followed by protein breakdown.

The advisability of using borax to preserve wastes of less organic matter content for subsequent B.O.D. analysis was still questionable. The dilution necessary for the B.O.D. determination of a concentrated waste should reduce the borax content to a non-toxic range. For in-

stance, in a waste of 1000 p.p.m. organic matter, only a one-milliliter sample is used per B.O.D. bottle, and no noticeable adverse effects on the B.O.D. of simulated dairy waste samples of that strength preserved by two percent borax should be expected. A compilation of results of tests made on various milk concentrations treated with two percent borax is presented in Table II. Three-day B.O.D. determinations were

TABLE II

EFFECT OF TWO PERCENT BORAX ON THE THREE-DAY B.O.D. OF VARIOUS MILK CONCENTRATIONS.

| Milk | B.O.D. | Ratio | Sample* | Borax** |
|---|---|---|---|---|
| p.p.m. | p.p.m. | B.O.D./milk | ml. | p.p.m. |
| 25 | 5.8 | 0.23 | 50 | 3333 |
| 50 | 15.4 | 0.31 | 20 | 1333 |
| 75 | 27.7 | 0.37 | 15 | 1000 |
| 100 | 46 | 0.46 | 10 | 667 |
| 200 | 104 | 0.52 | 5 | 333 |
| 400 | 197 | 0.49 | 2.5 | 166 |
| 750 | 380 | 0.51 | 1.5 | 100 |
| 1000 | 501 | 0.50 | 1.0 | 67 |

* Sample placed in B.O.D. bottle.

** Borax conc. in the B.O.D. bottle.

made, for it was believed any inhibitions due to borax would show up within that time. The ratio of B.O.D. to milk solids served as a measure of activity. At the higher concentrations of milk, this ratio was about 0.50. The ratio decreased significantly in samples of 75 p.p.m. milk and dropped markedly in more dilute waste. Control B.O.D. analyses of untreated simulated wastes gave the same B.O.D./milk ratios of 0.50. The approximate lower limit of borated milk concentrations measurable by the B.O.D. test appears to be 100 p.p.m. milk solids. A borax concentration of 667 p.p.m. in the B.O.D. bottle exerted little or no influence in this test series. Lower borax concentrations had no effect on bacterial growth. Tests made seven days later on the same wastes practically duplicated these findings.

An attempt to effect protection and obviate bacterial inhibition by using less borax in the lower concentrations of milk solids was made. Solutions of 100 to 400 p.p.m. milk solids were treated with 0.5, 1.0, 1.5, and 2.0 percent borax. The two percent borax-treated solutions gave the expected B.O.D. values; the 1.5 and 1 percent borax concen-

trations preserved the less concentrated milk solutions but were not effective in all cases, confirming previous odor and turbidity tests.

Field conditions were simulated in a final experiment in which quart-sized preserving jars were utilized. To each jar containing 800 milliliters of 100 to 400 p.p.m. milk solids 16 grams of borax (two percent) was added. Each jar was seeded with a generous inoculum of five milliliters aerated dairy sludge. The five-day B.O.D. and C.O.D. were determined immediately after seeding and again seven days later. Table III summarizes these data.

TABLE III

C.O.D. AND FIVE-DAY B.O.D. OF MILK SOLIDS INCUBATED WITH AND WITHOUT TWO PERCENT BORAX

| Incubation Days | Borax % | Milk Solids, p.p.m. 100 | 200 | 300 | 400 |
|---|---|---|---|---|---|
| | | C.O.D. | | | |
| 0 | 0 | 114 | 216 | 312 | 408 |
| 7 | 0 | 38 | 102 | 120 | 204 |
| 0 | 2 | 133 | 240 | 304 | 418 |
| 7 | 2 | 134 | 265 | 272 | 384 |
| | | 5-Day B.O.D. | | | |
| 0 | 0 | 68 | 130 | 201 | 250 |
| 7 | 0 | 5 | 14 | 11 | 31 |
| 0 | 2 | 67 | 118 | 165 | 229 |
| 7 | 2 | 65 | 127 | 190 | 246 |

Results showed agreement in C.O.D. and B.O.D. values between the controls and preserved samples for all four concentrations of milk solids tested. As evidenced by the B.O.D. data, no bacterial inhibition occurred even in the 100 p.p.m. milk solids samples which contained the greatest concentrations of borax. Included in this series was an unfed aerated sludge mixture of 432 p.p.m. C.O.D. which gave a B.O.D. of 179 p.p.m. both at the start and after seven days' storage in the presence of two percent borax, further emphasizing the non-inhibitory preserving ability of borax.

*Summary*

Samples of waste to be held for subsequent B.O.D. and C.O.D. testing may be preserved by the addition of two percent borax. This

amount sufficiently inhibited the activity of organisms in the waste so that reasonable results were obtained in postponed analyses. With dilution of the sample in the B.O.D. bottle, the bacteriostatic and bactericidal effects of borax were weakened or made negligible, so that no significant interference with the B.O.D. test occurred. To preclude interference with the activities of B.O.D. test organisms, final concentrations of borax in the B.O.D. bottle should be less than 1000 p.p.m. Borax had no measurable effect on the C.O.D. test.

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